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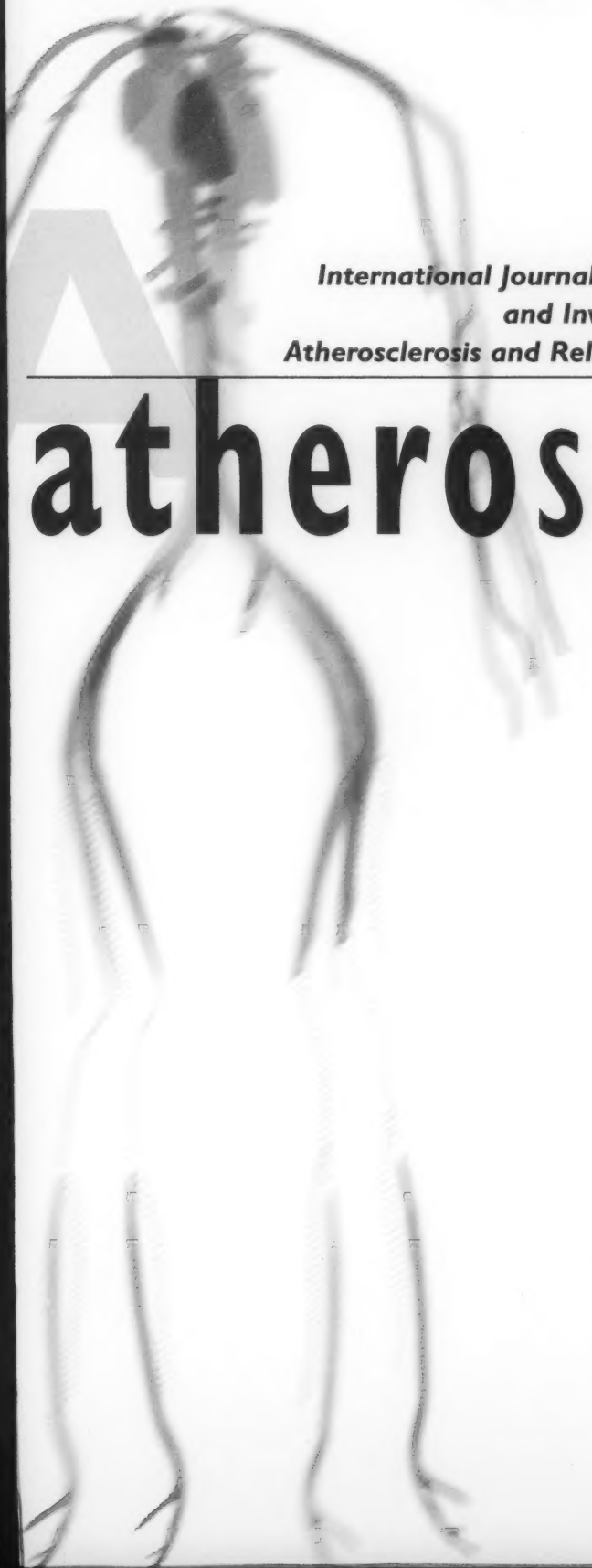

International Journal for Research and Investigation on Atherosclerosis and Related Diseases

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Affiliated with the International Atherosclerosis Society

Supplement
Atherosclerosis and Pentoxifylline: An International Symposium
Prague, 10-12 October 1996

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Atherosclerosis brings together from all sources papers concerned with research and investigation on atherosclerosis, its complications and related diseases, including: lipoprotein metabolism, arterial and vascular biology and disease, thrombosis, inflammation, disorders of lipid transport, diabetes and hypertension as related to atherosclerosis, and cardiovascular risk factors. The editors are also interested in clinical papers dealing with case studies of specific or general interest, new or unusual lipid syndromes, and the genetic basis and familial incidence of atherosclerosis and related diseases. High quality reports of controlled clinical trials of drugs or diets will be considered provided the paper deals with the mechanism of action of the drug or diet.

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Guest Editors
G. Schmid-Schönbein and S. Glagov

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Atherosclerosis 131 Suppl. (1997) S1

ATHEROSCLEROSIS

Introduction

Physicians and biomedical scientists, from many countries, gathered in Prague in October 1996 to discuss current knowledge of the mechanisms responsible for one of our most common disease processes atherosclerosis and current options for its treatment. Recent research has shed light on several mechanisms, including lipoprotein oxidation and endothelial shear stresses, which are only now being recognised as central components of the disease. Evidence of the pivotal role which appears to be assumed by oxidized lipids, together with other insights into the disease, such as the involvement of immunological responses, were presented during the first session of the meeting to give an overview of atherosclerotic mechanisms. The second session concentrated on the clinical manifestations of peripheral occlusive arterial disease (POAD), specifically intermittent claudication, and the often dramatic improvement in the quality of life which may be experienced by patients as a result of the use of effective treatments. Where appropriate, parallels were drawn between therapeutic approaches and the mechanistic evidence described in Session 1 to emphasise the cellular and molecular modifications by which symptomatic improvements may be achieved. This highlighted the types of therapy which are likely to prove most effective, both in clinical practice and economically, together with the approaches which offer most promise for future intervention.

The meeting was sponsored by Hoechst Marion Roussel.

Guest Editors
Geert Schmid-Schönbein and Seymour Glagov

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ATHEROSCLEROSIS

Cellular and molecular studies of atherogenesis

Russell Ross

Department of Pathology, University of Washington School of Medicine, Seattle, WA 98195-7470, USA

1. Introduction

The advanced lesions of atherosclerosis represent the culmination of a series of cellular and molecular events, involving replication of both smooth muscle cells and macrophages which have previously entered the artery wall. The interactions of these cells with T lymphocytes also in the lesion and the overlying endothelium can lead to a massive fibroproliferative response. Large amounts of connective tissue from smooth muscle cells form a fibrous cap. This covers the advanced lesion or fibrous plaque of atherosclerosis, deeper portions of which consist of macrophages, T lymphocytes, smooth muscle cells, connective tissue, necrotic debris and varying amounts of lipids and lipoproteins. Terminal events, e.g. myocardial or cerebral infarction, usually result from secondary changes in a fibrous plaque, which cause an occlusive thrombus to form. These changes include rupture of the lesion due to the forces created as a result of it encroaching into the vessel lumen.

2. The protective response

The cells involved in the earliest cellular events of atherogenesis are classical components of a specialized type of chronic inflammatory response which precedes the migration and proliferation of arterial smooth muscle cells. The first observable events include increased accumulation of lipid and lipoprotein particles beneath the endothelium, presumably due to increased transport and/or permeability of the lining endothelial cells. This is rapidly followed by the attachment and spreading of peripheral blood monocytes and T lymphocytes, particularly at branches and bifurcations of arteries. Adhesion occurs as a result of the formation of adhesive cell-surface glycoproteins on both the endothelium and the leukocytes. Thus, one of the earliest changes induced by hypercholesterolemia and hypertension appears to be altered endothelial permeability together with the adherence of leukocytes, i.e. the first phase of an inflammatory response.

Leukocytes migrate across the surface of the endothelium. This is facilitated by a series of molecules, including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). The leukocytes migrate between the endothelial cells. Chemotactic factors augmented by the endothelial or sub-endothelial cells then result in the localization of leukocytes within the sub-endothelial space, where they begin to accumulate within the intima.

The presence of oxidized low density lipoprotein (oxLDL) triggers the conversion of sub-endothelial monocytes into activated macrophages, which take up the modified lipoprotein particles and become foam cells. The formation and intimal accumulation of foam cells leads to the first ubiquitous lesion of atherosclerosis, the fatty streak, which is comprised of a mixture of lipid-filled macrophages and T lymphocytes, and is purely an inflammatory response. If the causative stimulus is maintained (e.g. hypercholesterolemia or other risk factors), the protective inflammatory response will also persist and can itself become deleterious to the cells of the artery wall.

This condition can lead to an expanded intermediate (or fibrofatty) lesion that may contain multiple layers of smooth muscle cells, macrophages, and T lymphocytes. Eventually, persistence of the conditions which induced the response causes the lesion to develop further. A fibrous cap forms, which covers proliferated smooth muscle cells and macrophages, necrotic cell debris, intracellular and extracellular lipid and newly-synthesised connective tissue. The advanced lesion or fibrous plaque can intrude into the artery wall. Resultant changes in the surface characteristics of the fibrous plaque may lead to formation of a thrombus which can cause sudden death or which can lead to further lesion progression and compromise of the local flow.

This sequence of events has lead to the 'response to injury' hypothesis of atherosclerosis, which suggests

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that what begins as a protective inflammatory response followed by the formation of a fibroproliferative response can, with time and with continued insult, become excessive. In its excess, the inflammation and fibrous tissue proliferation become the disease process.

3. Gene expression and intracellular signaling in lesion cells

Intercellular networking, which occurs among macrophages, T lymphocytes, endothelium and smooth muscle, is critical in determining lesion progression. For example, gene expression in macrophages can lead to the synthesis and secretion of many growth-stimulatory molecules, such as PDGF, fibroblast growth factor (FGF), heparin-binding epidermal growth factor-like growth factor (HB-EGF), monocyte colony-stimulating factor (M-CSF) and granulocyte-monocyte colony-stimulating factor (GM-CSF), as well as growth-inhibitory molecules, such as transforming growth factor β (TGF-β), interleukin-1 (IL-1) and tumor necrosis factor (TNF). However, the cytokines formed by macrophages, including TGF-β, IL-1 and TNF, can also induce secondary gene expression in smooth muscle cells for PDGF-AA and, in some cases, HB-EGF, thus inducing the cells to make growth-stimulatory molecules. Whether a lesion will progress, remain static or undergo regression may be partly a reflection of which of these genes is expressed in the different cell types.

Macrophage replication can occur through the action of CSFs formed by endothelium, smooth muscle cells or the macrophages themselves. Macrophage replication occurs during all phases of atherogenesis and may be as prominent a feature of lesion progression as is smooth muscle replication.

Chemotactic migration of medial smooth cells into the intima to form a neointima followed by the subsequent proliferation of these cells and formation of connective tissue by them are important activities in the formation and progression of lesions. Balloon catheter angioplasty, which has been used as a model in atherogenesis studies, causes rapid de-endothelialization and mural platelet thrombus formation followed by changes in the medial smooth muscle cells. Many of the muscle cells migrate through fenestrae of the internal elastic lamina into the intima where approximately half of them replicate and lay down large amounts of connective tissue matrix. In this second phase, the migration of smooth muscle cells from the media into the intima is largely induced by PDGF, which can be derived from the initial platelet thrombus or from PDGF-AA. The use of the balloon angioplasty model has shown that some components of chemotactic migration and replication of smooth muscle cells in the neointima are in part induced by PDGF, which may be both platelet- and smooth muscle cell-derived.

4. Summary

The lesions of atherosclerosis represent a protective, inflammatory-fibroproliferative response against the different agents which can cause the disease. With a chronic insult, the response may become excessive and thus constitute part of the disease process. The excessive response may be reversed, given sufficient opportunity for modification of the injurious factors. Approaches to modifying specific cellular interactions, growth-regulatory molecules or intracellular signaling molecules may afford opportunities for lesion prevention or regression. For further reading see [1,2].

Acknowledgements

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ATHEROSCLEROSIS

A critical look at the evidence for the oxidation of LDL in atherogenesis

Daniel Steinberg

Division of Endocrinology and Metabolism, Department of Medicine 0682, Basic Science Building Room 1080, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0682, USA

1. Initiation of the atherosclerotic lesion

There is now a consensus that the earliest atherosclerotic lesion, the fatty streak, begins under a structurally intact layer of endothelial cells and that the increased adherence of circulating monocytes to that endothelium is a very early response to hypercholesterolemia. These adherent monocytes penetrate the subendothelial space, where they are converted to 'foam cells', which contain most of the lipid in the fatty streak. A smaller number of foam cells arise from arterial smooth muscle cells. Low density lipoprotein (LDL) is the major source of the cholesterol accumulating in foam cells, but a growing body of evidence indicates that the LDL particle must undergo some form of modification before it can cause either foam cell formation or contribute to the atherogenic process in other ways.

2. Oxidative modification of LDL [1]

Oxidative modification is the best studied of the candidate modifications to LDL, but others may be equally important. Oxidized LDL (oxLDL) differs from native LDL in many ways. The more rapid uptake of oxLDL by macrophages, accounting for foam cell formation, was the first important difference to be described (Fig. 1). It was also quickly demonstrated that oxLDL is a chemoattractant for circulating monocytes and can thus play a role in their recruitment into a developing lesion. In addition, it has been shown that oxLDL inhibits the motility of tissue macrophages, which might exert a 'trapping' effect, leading to the accumulation of macrophages in a developing lesion. Of potential importance, especially in the later evolution of lesions, is the observation that oxLDL is cytotoxic to a number of cell types, including endothelial cells.

Over the past 5 years, a large number of additional properties of oxLDL which may enhance its atherogenicity have been described. For example, oxLDL is also a chemoattractant for T-cells, which comprise a significant proportion of the cells in atherosclerotic lesions. As another example, oxLDL stimulates the release of monocyte chemoattractant protein (MCP-1) from endothelial cells. Thus, oxLDL could, theoretically, exert both a direct and an indirect effect on the recruitment of monocytes from the circulation. A further property of oxLDL, not shared by native LDL, is its immunogenicity. Moreover, autoantibodies against oxLDL have been demonstrated both in rabbit and in human plasma. Whether these autoantibodies contribute to the pathogenesis of atherosclerosis, or are simply 'markers', is not yet certain. There are now at least 17 different ways in which oxLDL is, in principle, more atherogenic than native LDL, but the first question to be answered is, 'Does oxidation of LDL actually occur in vivo?'

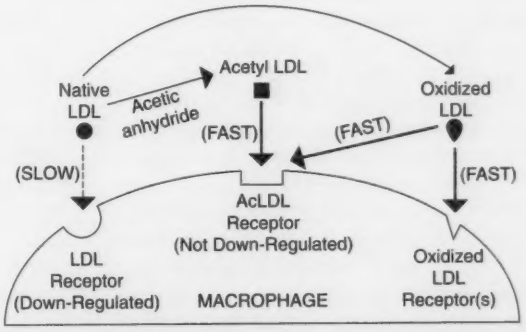

Fig. 1. Low-density lipoprotein (LDL) oxidation and relative speeds of uptake of oxLDL and native LDL.

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3. Evidence for the oxidation of LDL in vivo [2]

Four major lines of evidence demonstrate that oxidation of LDL does indeed occur in vivo:

1. OxLDL has been demonstrated in extracts of atherosclerotic lesions from both rabbit and human lesions.
2. Antibodies directed against oxLDL react with materials in atherosclerotic lesions of both humans and rabbits.
3. Autoantibodies against oxLDL have been demonstrated in both human and rabbit plasma, implying the presence of either oxLDL itself or some very closely related antigen.
4. Most importantly, it has been demonstrated that the administration of compounds that can prevent the oxidative modification of LDL significantly slows the progression of experimental atherosclerosis in rabbits, hamsters, mice and non-human primates. Twenty three studies have now been completed. In 16 of them, there was clear retardation of disease, ranging from 35 to 80%; two studies gave marginal results and five reported no effect. The studies have utilized four different antioxidants, greatly strengthening the conclusion that the effects observed were indeed due to the antioxidant properties of the compounds used (probucol, butylated hydroxytoluene, vitamin E and diphenyl-phenylenediamine). In some of these studies, the plasma cholesterol level was equal in the control and experimental groups and in none of them was there a large difference. It should be noted that this marked inhibition of atherogenesis occurred in the presence of cholesterol levels ranging from 600 mg/dl (in LDL receptor-deficient rabbits) to 2000 mg/dl (in cholesterol-fed rabbits).

4. The relevance of oxidized LDL to human atherosclerosis

A definitive statement on this issue awaits the completion of clinical intervention trials. However, several lines of evidence suggest that the human disease will respond in a similar fashion to that of animal models. Several epidemiological studies show an inverse correlation between dietary intake of antioxidant vitamins (vitamin E, vitamin C and β-carotene) and the risk of coronary artery disease. Some of these are cross-population studies and others are prospective studies based on dietary questionnaires. As mentioned above, oxLDL has been demonstrated in extracts of human atherosclerotic lesions. Furthermore, autoantibodies against oxLDL have been demonstrated in human plasma.

LDL isolated from the plasma of cigarette smokers immediately after they have smoked six cigarettes in a short period of time is much more susceptible to oxidative modification in vitro than control plasma LDL samples obtained prior to smoking. Whether or not this contributes to the increased susceptibility of smokers to atherosclerosis remains to be determined. Finally, it was reported in 1995 that high doses of supplemental vitamin E (400–800 IU/day) significantly reduced the incidence of non-fatal and fatal myocardial infarction in patients with established coronary disease [3]. However, it should be noted that other intervention studies, using β-carotene, have given negative results [4–7]. Administration of β-carotene does not protect human LDL against ex vivo oxidation; future trials will emphasize vitamin E.

5. Current strategy

Currently, there is insufficient justification for recommending antioxidant drugs or dietary supplementation by antioxidant vitamins (Table 1). While short-term studies show very few, if any, side-effects resulting from dietary vitamin E supplementation, there have been no studies of lifetime administration and, in the absence of direct evidence of benefit, no risk is acceptable. Therefore, until such evidence has been accumulated, we must continue to rely on dietary and drug-dependent management of LDL levels. These will also reduce levels of oxLDL in the artery.

6. Prospects

If the validity of the oxLDL hypothesis can be demonstrated by appropriate large-scale clinical intervention trials, we may expect an extensive study of natural antioxidants, an intensive re-evaluation of what constitute the best dietary patterns and, probably, a thorough study of new antioxidant compounds. Theoretically, an effective antioxidant regimen should be complementary to, and possibly synergistic with, a cholesterol-lowering regimen.

In addition to direct intervention to prevent LDL oxidation, a number of other possible interventions would, in theory, interrupt or slow atherogenesis. For example, the ultimate source of free-radicals involved in oxidation is probably intracellular. The precise enzymes involved in the generation of the free-radicals involved in LDL oxidation remain to be established. Several candidates have been identified, including 15-lipoxygenase and DPNH oxidase. The establishment of the importance of these candidates should allow the design of pharmacological interventions which modify the enzyme activity. With increasing understanding of the mechanistic steps in the atherogenic process, opportunities for intervention also increase. Examples may be agents which interfere with monocyte adhesion, or which abrogate the chemoattractant effects of MCP-1 and/or oxLDL.

Table 1
Reported clinical trials of antioxidants

Study	Result
Finnish ATBC Study [4] 29 000 male heavy smokers, 5–8 years. β-Carotene (20 mg/day) or vitamin E (50 mg/day) or both. Controls received neither antioxidant. Primary endpoint: cancer.	No effect on CAD events. Dose of vitamin E too low to protect LDL.
Swedish PQRST Trial [5] 274 hypercholesterolemic men and women. Cholestyramine $\pm$ probucol Primary endpoint: femoral artery stenosis.	No effect on femoral artery stenosis. Lesions in the femoral artery are far advanced; regression would be difficult to observe.
Physicians Health Study [6] 22 071 physicians randomized to β-carotene (50 mg q 2d) or placebo. Primary endpoint: cancer.	No effect on either cancer or CHD.
β-Carotene and Retinal Efficacy Trial (CARET) [7] 18 314 smokers or asbestos workers randomized to placebo or β-carotene (30 mg/day) and vitamin A (25 000 IU/day). Primary endpoint: lung cancer.	No reduction in lung cancer, nor in CHD.
Cambridge Heart Antioxidant Study (CHAOS) [3] 2002 patients with proven CHD randomized either to placebo or to vitamin E (400–800 IU/day). Primary endpoint: cardiovascular death or nonfatal infarction.	47% decrease in combined endpoints.

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ATHEROSCLEROSIS

Immunological response to oxidized LDL

Joseph L. Witztum [1]

Division of Endocrinology and Metabolism, Department of Medicine 0682, Basic Science Building Room 1080, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0682, USA

1. Introduction

Work by many laboratories has demonstrated that both early and extensively oxidized forms of LDL (oxLDL) contribute to atherogenesis in many ways (reviewed in [1,2]). In particular, oxLDL and some of its products are strongly pro-inflammatory. This inflammation is accompanied by both a humoral and a cellular immune response. Only recently has it emerged that the oxidation process renders LDL immunogenic, via the formation of a large number of neoepitopes on the modified LDL [3], and that oxLDL is undoubtedly an immunodominant antigen in the atherosclerotic lesion.

2. Humoral response in atherosclerosis

The peroxidation of the polyunsaturated fatty acids found in LDL results in the generation of highly reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). In turn, MDA and 4-HNE form adducts with lysine residues on apo B (the protein component of LDL) to produce immunogenic epitopes. Antisera and monoclonal antibodies specific to MDA-lysine and 4-HNE-lysine immunostain atherosclerotic lesions, but do not stain normal artery [3]. Furthermore, autoantibodies to these epitopes are present in the plasma of animals and humans. The antibody titers to various oxLDL epitopes in Apo E-deficient mice, which develop severe atherosclerosis as a result of marked hypercholesterolemia [4] are shown in Fig. 1. These titers are considerably elevated compared to those found in C57BL/6 mice, which are normal. In addition, preliminary studies have suggested that patients with extensive atherosclerosis have relatively higher antibody levels [5], but much more work is needed.

Extensive accumulation of IgG occurs within atherosclerotic lesions, some of which is oxLDL-specific and is present in the form of immune complexes with oxLDL. Autoantibodies to oxidatively-modified phospholipids have also been found [6]. It seems likely that oxidation of numerous components of the artery wall may prove immunogenic and stimulate autoantibody formation.

3. Cellular immune response in atherosclerosis

T-lymphocytes make up 10 to 20% of the cells in early atherosclerotic lesions. Many of these cells are activated, expressing gamma interferon (IFNγ), as demonstrated by the expression of HLA-DR by vascular cells within the atherosclerotic lesion [7]. Studies by Hansson and colleagues have demonstrated that 10% of a population of T-cells cloned from human carotid atherectomy specimens reacted specifically with oxLDL [8]. Of the T-cells which accumulate, at least within established lesions, there is a predominance of the Th_1 type, which primarily secrete IFNγ.

4. The role of the immune system in atherosclerosis

The role of the immune system in atherosclerosis is currently unclear. Certainly, in atherosclerosis following cardiac transplantation, the immune response appears to be of major significance [9], while in more common cases of atherosclerosis, occurring as a result of hypercholesterolemia, increasing evidence supports an important role for both humoral and cell-mediated immunity. Evidence of a protective as well as a pathological effect of the immune response has been demonstrated in animal models of atherosclerosis. For example, immunization of hypercholesterolemic rabbits (WHHL) with homologous MDA-modified LDL led to

[1] Tel.: +1 619 5344347; fax.: +1 619 5342005.

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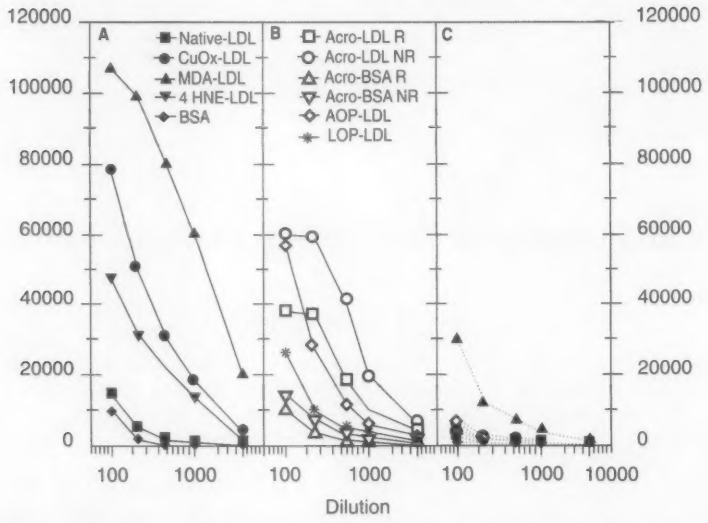

Fig. 1. Dilution curves of IgM autoantibodies in pooled sera of two apo E-deficient mice to various epitopes of oxidized LDL, compared with similar curves from age-matched C57BL/6 controls [4]. Reproduced with permission, Copyright 1995, National Academy of Sciences, USA.

a reduction in the rate of progression of atherosclerosis (Fig. 2) [10]. Similar results have been reported by other groups [11]. One hypothesis (yet to be explored) to explain this observation is that immunization leads to an increase in the plasma clearance rate of minimally-modified LDL. The relative importance of the pathological or protective immune responses in atherosclerosis remain to be elucidated.

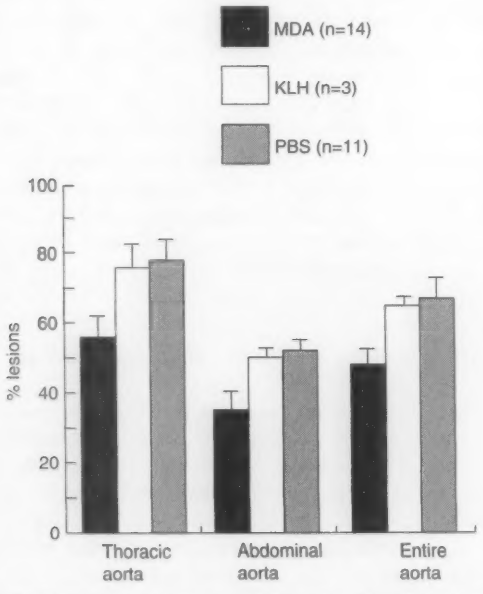

Fig. 2. Percentage of aorta covered by atherosclerotic lesions in WHHL rabbits 6 months after immunization with homologous MDA-modified LDL (MDA) or keyhole limpet hemocyanin (KLH) or saline (PBS) [10]. Reproduced by copyright permission of the American Society for Clinical Investigation.

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Atherosclerosis 131 Suppl. (1997) S13–S14

ATHEROSCLEROSIS

Mechanical determinants of plaque modeling, remodeling and disruption

S. Glagov *, H.S. Bassiouny, Y. Sakaguchi, C.A. Goudet, R.P. Vito

Departments of Pathology and Surgery, University of Chicago, Chicago, IL and Department of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, USA

1. Introduction

The stability of the blood vessel wall and adequate perfusion of the tissues are maintained by structural adaptations to changes in wall shear and tensile stresses. These readjustments take the form of morphological reconstructions of vessel wall dimensions, composition and configuration. Altered flow results in adjustments to the diameter of vessels, which tend to restore wall shear stress to baseline values of 15–20 dyn/cm^2, while changes in vessel diameter or in fluid pressure produce altered mural tensile stresses which, in turn, generate corresponding changes in wall thickness and composition.

During early development and growth, the proliferation of smooth muscle cells tends to correspond to an enlargement of the vessel diameter and an increase in wall volume, while matrix accumulation corresponds largely to tensile stress. After growth, the media and intima may both participate in these reactions. In conditions of decreased flow, intimal cell accumulation narrows the vessel lumen, thereby increasing wall shear stress until the baseline shear stress is restored. The intima then develops cellular and matrix features of the media.

2. Plaque remodeling

Remodeling as a result of changing mechanical and metabolic states occurs at all stages of the atherosclerotic process. Proteolytic, organizational and matrix biosynthetic responses result in erosion and/or sclerosis both beneath and within the plaque [1]. These responses produce more or less characteristic stratifications within the atherosclerotic lesion, including the formation of a subendothelial fibrous cap and outward bulging and sequestration of the plaque, which consequently isolates potentially thrombogenic components from the arterial lumen. This preserves an approximately circular or slightly oval lumenal contour, and so favors stable laminar blood flow. Compensatory enlargement of the artery may also occur to preserve an adequate lumenal cross-sectional area for extended periods [2]. Flow field properties related to the geometric configuration of the vessel correlate significantly with plaque localization [3], while tensile stress distribution is a major determinant of plaque organization and matrix production. Changes in the composition and configuration of advanced plaques are associated with the control of risk factors. The formation of new plaque structures on older plaques, direct intervention and disruptive complications all elicit secondary proliferative healing and remodeling responses.

A comparison of plaque evolution at the carotid bifurcation, in the coronary arteries and in the distal aorta and peripheral arteries reveal differences which are likely to reflect differences in the time of onset of the lesions, in the hemodynamic and tensile stress environments and in differential local responses to specific clinical risk factors, such as smoking, diet or lack of exercise. For example, in the coronary arteries marked stenosis is important in producing symptoms, but plaque disruption may be significant at less stenotic stages. Although such an effect is difficult to assess in plaques at the carotid bifurcation, since clinical detection and therapeutic endarterectomy are associated mainly with highly stenotic plaques [4], the resulting specimens do provide material for the identification of specific features associated with ischemic attacks or stroke, such as necrotic plaque cores, calcifications and thrombus deposition.

* Corresponding author. Tel.: +1 773 7026228; fax: +1 773 7023778.

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Table 1
Morphological correlates of plaque instability

Factors associated with disruption
Large plaque with marked stenosis
Juxtaposed regions of contrasting composition (calcification, necrotic core, fibrosis, hematomas)
Lumen irregularities and asymmetry (thromboses and cavitations)
Focal thinning or defects of the fibrous cap (erosion, inflammation, neoformation of atherosclerosis)
Proximity of the necrotic core to the fibrous cap or lumen surface
Features implying resistance to disruption
Uniform plaque fibrosis on cross-section
Circular, regular lumen contour
Demarcated fibrous cap of uniform thickness (with absence of focal erosion, inflammation and proximity of necrotic core)

Table 2
Clinical evaluation of advanced plaques: problems arising in establishing reliable morphological criteria

Geometric factors
Circumferential asymmetry of plaque localization
Axial and circumferential variation in plaque size and composition
Plaque evolution
Uncertainty of plaque evolution
Occurrence of focal disruptions in apparently stable plaques
Often small, inapparent nature of sites of actual or potential disruption
Uncertain nature of lumen irregularities
Technological
Current limits (in specificity and sensitivity) of imaging methods

Extensive regions of low density or echo-lucency in large plaques are considered to be indicative of vulnerability to plaque disruption. However, a recent comparative study of asymptomatic and symptomatic stenotic specimens suggested that the proximity of the necrotic lipid core to the lumen or the fibrous cap, rather than the actual size of the lipid core, may be a more reliable indicator of which plaques caused symptoms (Table 1).

3. Determinants of stress distribution within the plaque

Conditions under which mechanical factors induce disruptions in susceptible fragile plaques is not well understood. The use of finite element analysis in the study of stress distribution in plaque cross-sections from carotid bifurcation endarterectomy specimens has revealed that stresses are greatest within the fibrous cap [5]. In addition, proximity of the necrotic lipid core or macrophages to the fibrous cap, particularly to thinned regions of the cap, significantly increases both the peak stress and the stress gradient. Movement of the lipid core closer to the fibrous cap has been demonstrated to increase the maximum stress by over 60%, while the presence of discrete calcifications increased the peak stress by up to 70% and displaced its focus to within the plaque. Thus, the distribution of plaque constituents, particularly the necrotic core and calcification regions, appears to determine both the location and the magnitude of the peak stress within the plaque and therefore the propensity for plaque rupture.

The anticipation of clinically significant precursors of plaque disruption is limited by uncertainty regarding the predictability of plaque evolution, by the often focal nature and small size of disruptive changes and by inadequacies in resolution offered by current investigation techniques (Table 2). The development of methods for the sequential clinical examination and reconstruction of complete plaques, with corresponding histopathological validation, holds the promise of establishing criteria for discerning both plaque composition and the potential susceptibility of plaques to disruption [6]. This may allow the timely prediction of potential plaque rupture, thereby permitting intervention at a pre-critical stage.

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Atherosclerosis 131 Suppl. (1997) S15–S17

ATHEROSCLEROSIS

Mechanisms involved in endothelial responses to hemodynamic forces[1]

Peter F. Davies [2]

Institute for Medicine and Engineering, University of Pennsylvania, 117 Hayden Hall, 3320 Smith Walk, Philadelphia, PA 19104-6392, USA

1. Introduction

Blood flow in arteries, arterioles and pre-capillary vessels generates hemodynamic shear stresses. These typically range from 0 to 50 dyn/cm^2 and vary in magnitude, frequency and direction at the endothelial surface throughout the cardiac cycle [1]. In addition to flow-mediated vasotonic control, hemodynamic forces play a pivotal role in atherosclerotic lesion development in humans. Lesions tend to develop in areas where there is a deviation from unidirectional laminar blood flow, typically near branches, bifurcations, regions of arterial narrowing and curvature. Mechanisms responsible for the transmission and transduction of hemodynamic information from the blood to the underlying vessel wall reside in the endothelium [2] and considerable progress has been made in determining the signaling events at the cell surface(s) and the gene regulatory elements associated with cellular adaptation to mechanical stress [3,4].

Endothelial cells are capable of altering their structure and mechanical properties. When there is a net direction to the flow force, endothelial cells align in the direction of flow [5,6], but in areas of turbulence, the cells fail to align [7]. These changes result in the generation of intracellular stress. Tension is generated when the cytoskeleton interacts with other regions of the cell, particularly at sites of adhesion to the sub-endothelial matrix [8], the nucleus [9] and neighboring cells [10]. The intercellular tension changes to equalize external force when external forces are loaded onto the cell during flow.

2. Topography of the lumenal endothelial surface

The hemodynamic force-cell interactions begin at the cell surface. Cell geometry, particularly surface topography, influences the magnitude and localization of hemodynamic forces acting at the endothelial surface and affects their transmission throughout the cell. Previous subcellular analyses of flow forces addressed the mechanisms of force transmission and transduction into cellular responses by modeling the endothelial monolayer and estimating the influence of its wavy surface upon local flow forces [11]. More recently, high resolution, real-time images of the continuous geometry of the lumenal surface of living endothelial cells have been made using atomic force microscopy (AFM) [12]. These studies revealed significant changes in the height of the endothelial monolayer ($< 6\ \mu$m) when cells aligned with flow to give a streamlined cell surface (Fig. 1). In addition, localized changes of topography of individual endothelial cells were observed which have implications for the

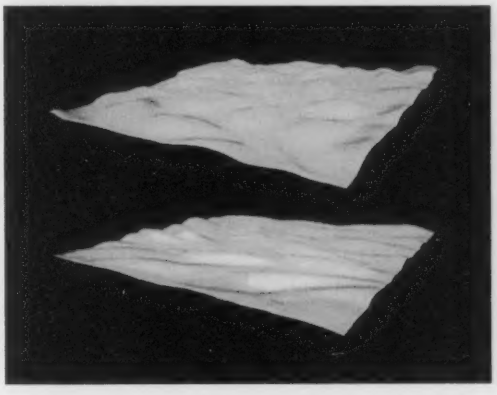

Fig. 1. Computer-generated images of blood-vessel endothelium, showing non-aligned endothelial cells in the absence of flow-induced shear stress (upper image) and flow-aligned endothelial cells (lower image). Adapted from [13], with the permission of the American Physiological Society.

[1] Condensed from: Davies PF. Physiol Rev 1995;75:519–560.

[2] Tel.: +1 215 8984647; fax: +1 215 5736815

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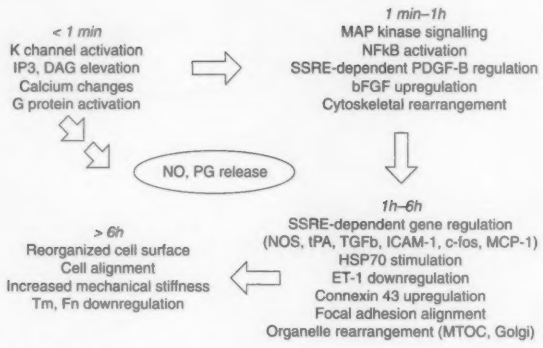

Fig. 2. Effects of shear stress on blood vessel endothelium [3]. Reproduced with the permission of the American Physiological Society.

intracellular stress distribution. Spectral element analyses have been conducted to simulate flows and to determine shear-stress distribution over endothelial surface geometries (defined by AFM) of living endothelial cells [13]. Flow perturbations due to the undulating surface produce large shear stress gradients, but reorganization of the endothelial surface in response to prolonged exposure to steady flow results in significant reductions in the peak shear stresses and shear stress gradients compared to no-flow control cells [12,13]. These studies have demonstrated that there are microscopic departures from a flat endothelial boundary due to the presence of endothelial cells that, in turn, cause a localized perturbation of the macroscopic flow field.

For a given endothelial cell, lumenal shear stress varies within the macroscopic flow field as a function of the cell surface geometry; cells in certain morphological configurations being likely to be exposed to much lower shear stress gradients than neighboring cells. In flow-aligned cells for example, there is approximately a 40% decrease in average shear stress gradient compared with no-flow control cells. A detailed analysis of the upstream/downstream symmetry of the cell surface in vitro may provide useful insights into individual cell sensitivity to shear stress. Preliminary results from AFM images demonstrate very similar morphology between endothelial cells in vivo and cultured cells [14]. This has led to a proposal that the detailed cell surface topography contributes to the stress response in vivo.

One of the most striking consequences of flow-mediated endothelial realignment is increased resistance of the cell surface to deformation [15]. This is facilitated by the formation, after exposure to flow, of realigned arrays of bundles of cytoskeletal filaments immediately below the lumenal plasma membrane.

3. Intracellular signaling

Early responses to shear stress (Fig. 2) include rapid changes in ionic conductance, inositol phosphate generation, cyclic GMP concentration, activation of GTP binding proteins and, in some cases, the elevation of intracellular free calcium. Inwardly-rectifying endothelial K^+ and Cl^- ion channels, rapidly activated by shear stress, facilitate membrane hyperpolarization followed by depolarization. An endothelial inward-rectifying channel has recently been cloned, but it remains to be seen if this channel is flow responsive. The release of neurotransmitters within a few seconds of flow through a microcarrier bed of confluent endothelial cells has also been reported and within 1 min of flow initiation, the release of substantial amounts of the potent vasoactive agents prostacyclin and nitric oxide also occurs. These acute responses can be rapidly controlled and their vasoactive effects quickly reversed, a desirable evolutionary advantage. If the hemodynamic forces are sustained, however, adaptive responses are initiated. The induction of some early response genes is detected (c-*myc* after several min, c-*fos*, c-*jun* within 2 h) and there is activation of NFkB transcriptional factor complex [16]. Since NFkB binding sites are found in many genes, this system may play a role in gene regulatory responses to flow. A shear stress response element (SSRE) has been identified in the promoter of the platelet-derived growth factor B gene (PDGF-B gene) and this element appears to bind NFkB and possibly other unknown transcription factors [4]. The same motif is found in several other genes known to be shear stress responsive, including tissue plasminogen activator, c-*fos*, c-*jun*, monocyte chemotactic protein 1, transforming growth factor β_1 and intercellular adhesion molecule 1. Furthermore, the transfection of a reporter gene containing SSRE into non-responsive cells results in flow-inducible reporter gene activity. Other apparent SSRE sites have recently been uncovered. Identification of SSRE binding molecules should reveal a key regulatory mechanism of mechanically-induced gene responses.

4. Cell attachment sites respond to flow

Endothelial cells, like other anchorage-dependent cells, form discrete regions of contact with the underlying extracellular matrix, called focal adhesion sites [8]. When subjected to directional shear stress, membrane remodeling occurs at adhesion sites, with a preference for the direction of flow [17], but without a change of location of cell boundaries or organelle distribution. Cell adhesion remains unchanged. These live cell studies demonstrate that the interactions between the ablumenal cell surface and the substratum are

flow-responsive and are mediated by mechanical transmission from the lumenal to the ablumenal surface via stress fibers anchored at focal adhesion sites. The mechanisms by which membrane attachment to the substratum remodels during spontaneous and flow-induced reorganization remain unknown. However, more information is emerging concerning the proteins localized to focal adhesions, particularly the phosphorylation states of a number of integrin-associated proteins, some of which are kinases [18] and whose activities may represent signaling pathways associated with mechanotransduction at these sites.

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Atherosclerosis 131 Suppl. (1997) S19–S21

ATHEROSCLEROSIS

Phagocyte implications in advanced atherosclerosis

Dominique de Prost, Jacques Hakim *

Faculte de Médecine Xavier Bichat, Laboratoire d'Hématologie et de l'Immunologie, 46 Rue Henri Huchard, 75877 Paris Cedex 18, France

1. Phagocyte accumulation

Epidemiological data reviewed by Belch [1] suggest that leukocyte as well as neutrophil counts are powerful, consistent predictors of cardiovascular events. An increase correlates with the risk of coronary artery disease and with both the incidence and severity of thrombotic stroke. Total leukocyte count is also predictive of an increase in the amputation rate in patients with critical lower limb ischemia.

An example of the more direct involvement of phagocytes in advanced atherosclerosis is the dynamic accumulation of monocytes, macrophages and lipid-laden macrophages ('foam cells') in the atherosclerotic plaque (Fig. 1). The turnover of cells in the plaque is dependent on the adhesion of circulating monocytes to the endothelium covering the plaque core, followed by their migration. Adhesion molecules expressed by the endothelium attach to adhesion molecules expressed by monocytes [2,3]. The expression of the endothelial adhesion molecules is induced by various stimuli, including reactive oxygen species [4]. Endothelial adhesion molecules include E- and P-selectins, as well as intercellular adhesion molecule-1 (ICAM-1). Adhesion molecules on the monocytes include sialylated or sulphated carbohydrates and β2-integrins. The migration of the monocytes across the endothelium requires a further agent, platelet endothelial cell adhesion molecule-1 (PECAM-1), which is expressed on the monocytes and lateral faces of endothelial cells and induced by the release from the plaque cells of chemoattractants, such as lipid peroxides, chemokines or leukotriene B4.

In the plaque, the adherent monocytes change to macrophages and ingest lipid peroxides. This activates the cells and leads to their development into foam cells. Macrophages within the lesion are also activated by their adhesion to the plaque and by soluble factors such as monocyte chemoattractant product-1 (MCP-1) and cytokines, including monocyte colony-stimulating factor (M-CSF). Among other sources, MCP-1 is synthesized by endothelial cells stimulated by mechanical strain and thrombin-stimulated vascular smooth muscle cells, while cytokines are produced by T lymphocytes. Macrophage autoactivation also occurs, secondary to their own production of MCP-1 and cytokines.

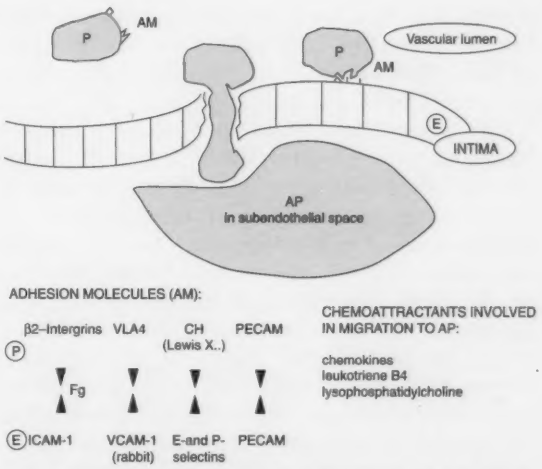

Fig. 1. Adhesion of phagocytes (P) to blood vessel endothelium (E), orientated transmigration and recruitment into the atherosclerotic plaque (AP). Adhesion molecules (AM) expressed on P are β2-integrins, carbohydrate moieties (CH) and very late antigen 4 (VLA4). Intercellular adhesion molecule 1 (ICAM-1) link is strengthened by fibrinogen (Fg). VCAM-1 is for vascular adhesion molecule-1. Platelet endothelial cell adhesion molecules (PECAM) are expressed on both P and E, and used in transmigration which is oriented by chemoattractants.

* Corresponding author. Tel.: +33 1 40258521; fax: +33 1 40258853

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2. Role of oxidized LDL

Activated macrophages generate numerous molecules which can modify the vessel wall. Macrophages can effect lipid peroxidation, via the production of reactive oxygen species. This is associated with an increase in oxidized low-density lipoprotein (oxLDL) and a decrease in high-density lipoprotein, with a resulting accumulation of cholesterol in the plaque. Reactive oxygen species (ROS) increase arachidonic acid metabolism, endothelin generation and the expression of adhesion molecules by the endothelium, while decreasing the production of endothelium-derived relaxing factor (EDRF). Endothelial function is also modified by ROS, with an increase in vascular tone and hyperpermeability, allowing large-scale diffusion of plasma molecules, such as coagulation factors. Activated macrophages also generate growth factors which induce the proliferation of vascular smooth muscle cells, thickening the vascular wall. Most importantly, activated macrophages release metalloproteinases and other proteases which contribute to the degradation of the plaque matrix, leading to aneurysm formation and plaque rupture. These effects occur very slowly, the plaque typically evolving over several decades. The pathological changes are, to some extent, compensated for by protective mechanisms effected by the macrophages themselves. For example, macrophages participate in the active elimination of cholesterol crystals from the plaque via an M-CSF-induced increase in enzymes which metabolize lipids and cholesterol, the formation of HDL, the generation of gamma interferon and the formation of discoidal and vesicular lipoproteins. Macrophages can also leave the plaque, loaded with lipids and cholesterol.

The lipoperoxides which accumulate within the macrophages are cytotoxic. These kill the cells and are thereby released into the core of the plaque, which is prone to rupture, partly as a result of the action of enzymes released by the dying cells. This event marks the transition from the chronic 'silent' phase of atherosclerosis to the acute, life-threatening stage. One complication of plaque rupture is vessel thrombosis. Phagocytes are important factors in the thrombotic process, producing tissue factor (TF) which induces the coagulation cascade. In cells of the vessel wall, TF is highly inducible by a variety of agents, including TNF, endotoxin, interleukin-1, α-thrombin and ROS. Similarly, in monocytes TF production is stimulated by a variety of inflammatory mediators, such as ROS, endotoxin, C-reactive protein and by adherence to endothelial cells. In arterial smooth muscle cells, growth factors, angiotensin II and α-thrombin are all capable of inducing TF expression.

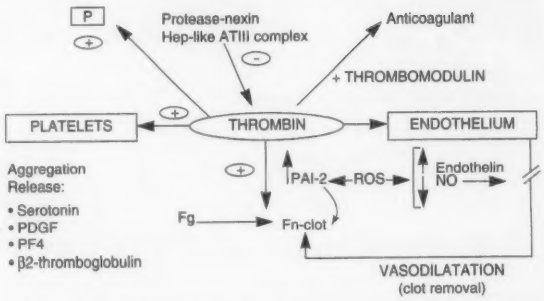

Fig. 2. Effects of thrombin in atherosclerosis. Effects of thrombin are designated by an arrow and a + or − sign, respective of whether the result is an activation or an inhibition. An interrupted arrow (–//–) means that the effect is inhibited. Abbreviations used are: P, phagocyte; Hep-like ATIII, heparin-like antithrombin III; ROS, reactive oxygen species; PAI2, plasminogen activator inhibitor 2; Fg, fibrinogen; Fn, fibrin; PDGF, platelet-derived growth factor; PF4, platelet factor 4.

3. Tissue factor in thrombogenesis

Evidence to implicate TF in atherosclerotic thrombus formation continues to accumulate. Intimal endothelial cells do not contain detectable TF mRNA or TF protein and, in medial smooth muscle cells, TF protein is present only at very low levels. However, adventitial fibroblasts sequestered from the circulating arterial blood give strongly positive immunostaining and mRNA hybridization for TF. Furthermore, endothelium from plaques from carotid endarterectomy specimens are negative for TF mRNA and protein in the endothelium, whilst a positive response was obtained in cells presumed to be smooth muscle cells, and in foam cells and monocytes adjacent to cholesterol clefts and in the necrotic core of the plaque [5]. Therefore, TF induction in the plaque could contribute to the hyperthrombotic state of atherosclerotic vessels. The frequent expression of TF in de novo lesions of patients with unstable coronary syndromes also suggests that TF plays a central role in the pathogenesis of this disease process and implies that such patients may benefit from the use of TF pathway inhibitors [6].

The coagulation cascade, induced by TF, leads to the generation of thrombin that, in addition to the formation of fibrin clots, acts on several components of the atheromatous vessel. Thrombin is chemotactic for monocytes and neutrophils, activates these cells and stimulates platelet aggregation (Fig. 2). Whereas in normal vessels thrombin induces vasodilation, in atherosclerosis it decreases endothelial production of nitric oxide and increases the production of endothelin-1 and, consequently, vasoconstriction. Through their proteolytic and fibrinolytic effects, neutrophils and monocytes may be beneficial, by lysing a formed clot.

4. Conclusions

Phagocytes appear to play a major role in the evolution of the atheromatous plaque, the progression from a chronic to an acute phase (i.e. plaque rupture) and in the formation and lysis of thrombi. Therefore, the pharmacological manipulation of phagocyte action by anti-inflammatory agents may have beneficial effects.

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Atherosclerosis 131 Suppl. (1997) S23–S25

ATHEROSCLEROSIS

Mechanisms of leukocyte activation in the circulation

Geert W. Schmid-Schönbein *, Benjamin W. Zweifach, Fariborz Moazzam

Department of Bioengineering, Room 4100, Engineering Building, Unit 1, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0412, USA

1. Introduction

The cells of the cardiovascular system may be found both in a relatively quiescent, essentially inactive state and also in a state in which several forms of activation are manifest, including (depending on the type of cell involved) oxygen free-radical formation, expression of adhesion molecules and cytokines, degranulation and the projection of pseudopodia (Fig. 1). Although each of these signs of activation are integral to physiological functions, abnormal generalized circulatory cell activation occurs during some forms of cardiovascular disease and may even be found associated with conditions causing cardiovascular risk prior to signs of overt disease.

2. Forms of cell activation and their consequences

The projection of pseudopodia by circulating leukocytes [1], platelets or endothelial cells [2] has an immediate profound effect on local perfusion, particularly in the capillary segment of the microcirculation. Leukocytes with pseudopodia have reduced deformability and so can reduce blood rate even in the normal circulation [3] and are likely to become trapped in the microcirculation [4].

The expression of surface adhesion molecules, such as the selectins, integrins and members of the immunoglobulin superfamily, represents another aspect of cell activation, important in immunology but also of significance in the progression of cardiovascular disease [5]. These molecules mediate a cascade of leukocyte/platelet/endothelial interactions accompanied by oxygen free-radical formation, leukocyte migration and degranulation [6,7]. Several adhesion molecules are stored in intracellular granules and become incorporated into the plasma membrane on degranulation. In lysosomes, degranulation also leads to the release of proteolytic enzymes, possibly accompanied by marked parenchymal cell damage.

In models of circulatory challenge, the degree of cell activation influences the prognosis. In hemorrhagic shock, for example, animals with low activated leuko-

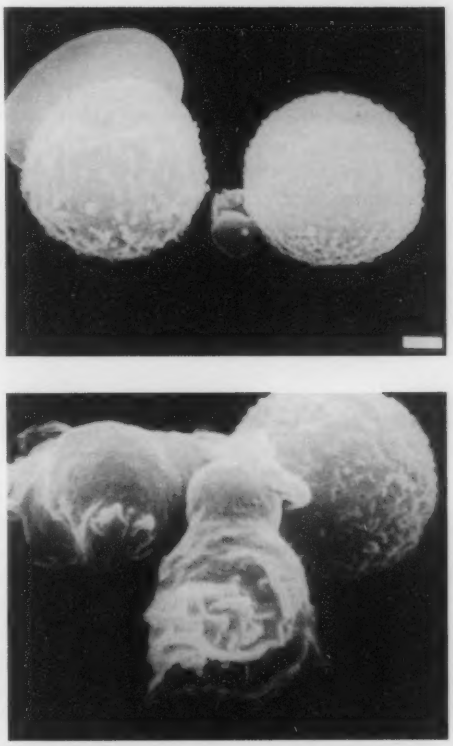

Fig. 1. Scanning electron micrographs of inactive (upper image) and activated (lower image) leukocytes. A pseudopodium extends from the activated cell; the inactive cell lacks membrane projections.

* Corresponding author. Tel.: +1 619 5343852; fax: +1 619 5346896.

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Table 1
Mechanisms of granulocyte/monocyte activation

1	Activating agents, e.g. complement products, LTB_4, PAF, LPS, interleukins, TNF, oxidized lipids and cholesterol, etc
2	Depletion of deactivators, e.g. adenosine, albumin, nitric oxide, corticosteroids.
3	Shear stress
4	Cell contact activation, e.g. on endothelial cells expressing selectins, ICAM-1

cyte counts prior to the reduction in blood pressure have a higher chance of surviving the period of low flow [8].

3. Manifestations of cell activation in humans

Most measurements of cell activation in the human circulation are limited to circulating leukocytes and platelets, although activation may also involve other cell types. In stroke [9], myocardial infarction [10], claudication [11] and venous disease [12], circulating leukocytes exhibit significantly elevated levels of activation. A similar picture of cell activation may be observed in smokers with a significant risk for cardiovascular disease [13]. In addition, the ability to activate naive leukocytes from smokers suggests that the plasma of smokers contains an activating factor which is likely to reach all organs, thereby potentiating the widespread organ complications which often accompany smoking.

In animal models, the majority of activators affect both neutrophils and monocytes to a similar degree. However, the activation in humans may be more differentiated: neutrophil activation may lead to acute inflammation and tissue injury, while monocyte or T lymphocyte activation may lead to chronic atherosclerosis as well as other chronic cardio-vascular complications.

4. Activators versus deactivators

In addition to the traditional activating factors, leukocytes may be activated by the depletion of deactivating factors (Table 1). Activation is often accompanied by integrin-mediated cell adhesion and free radical formation. There is extensive evidence that fluid shear-stress also influences both platelet and endothelial cell function [14]. Transient shear-stress activates endothelial cells, as does membrane contact. Chronic shear-stress induces stress fiber formation and is accompanied by the downregulation of many cell functions, including those of leukocytes.

5. Influence of shear-stress on leukocyte activation

The majority of circulating leukocytes are relatively passive, in contrast to the active amoeboid motion and adhesion to the endothelium required for their migration from the bone marrow. Recent evidence suggests that application of a mild fluid shear-stress (approximately 1 dyn/cm^2) to leukocytes in vitro leads to instantaneous retraction of pseudopodia and is also associated with F-actin formation and cytoplasmic stiffness. This suggests that fluid shear-stress of the magnitude encountered in the circulation is an important regulator of leukocyte activation [15].

6. Conclusion

The elucidation of the mechanisms underlying generalized cell activation in the cardiovascular system may reveal novel approaches for intervention in several forms of cardiovascular disease.

Acknowledgements

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Atherosclerosis 131 Suppl. (1997) S27–S28

ATHEROSCLEROSIS

Pentoxifylline: its influence on the interaction of blood cells with the vessel wall

Dirk Seiffge [1]

Hoechst Marion Roussel, TA Rheumatology, PO Box 3540, 65174 Wiesbaden, Germany

1. Basis of atherosclerosis

The pathophysiology of vascular disease is characterized by an impairment of the nutritive microcirculation arising from impaired blood flow and cellular function. While erythrocytes and platelets, in particular, are implicated in atherogenesis, it has become evident that leukocytes also play an important role in the nonreflow phenomenon in ischemic tissue under pathophysiological conditions of low blood flow or low pressure gradients [1].

The development of atherosclerosis in large arteries is determined by two factors: the vessel wall structure and the vessel contents (e.g. blood cells, lipoproteins and cholesterol). These factors are linked to the accumulation of intimal lipid and the development of fibrous changes within the intimal layer. The non-Newtonian nature of blood, which generates complex hemodynamic forces such as shear-stress, high- and low-velocity gradients and pressure gradients, also plays a role in atherogenesis, as does the gross geometry of the vessels. Areas in which laminar flow is disturbed and vortices and reverse-flow are found, e.g. at vessel bifurcations and sharp curvatures, appear to be particularly prone to atherosclerotic lesion development [2].

2. Role of oxidized LDL on cell function in atherogenesis

Oxidized low-density lipoprotein (oxLDL) has been identified as a potent chemotactic agent, instrumental in the migration of leukocytes into the subendothelial space during early atherogenesis [3,4]. Interestingly, oxidized LDL is far more atherogenic than native LDL. Using a skinfold chamber model in hamsters and mice, it has been demonstrated that oxLDL, but not native LDL, elicits rolling of leukocytes and their adhesion to the microcirculatory endothelium within minutes of oxLDL injection [5]. Such adhesion is now recognized as an early marker of an inflammatory response. This adhesion of leukocytes (neutrophils, monocytes and lymphocytes) to blood vessel walls is promoted by endothelial adhesion molecules. A number of inflammatory mediators and cytokines have been described that promote leukocyte adhesion and also induce the extravasation of plasma macromolecules [6]. Mediators elaborated by leukocytes, including prostanoids, leukotrienes and platelet-activating factor, may affect the intrinsic vascular wall and so contribute to lesion formation.

3. Action of pentoxifylline (PTX)

Pentoxifylline (PTX) improves microcirculatory perfusion of both the peripheral and cerebral vascular beds. The underlying mechanism of action appears to be related to an increase in erythrocyte deformability, a reduction in blood viscosity, a reduction in plasma fibrinogen concentration and an improvement in platelet and leukocyte function. In addition to its role in modifying specific atherogenic properties within blood cells, PTX also elicits beneficial endothelial responses, enhancing fibrinolysis and stimulating the anti-aggregatory activity of prostacyclin, as measured by an increase in platelet cAMP (PTX inhibits phosphodiesterase activity and stimulates cAMP production in the platelets) [7]. Furthermore, PTX exhibits favourable effects on endotoxin-induced shock or inflammation [8].

In investigating these vessel wall effects, animal models have been used to study the effects of PTX on the susceptibility of the wall to laser-induced thrombus formation and to atherosclerotic plaque development.

[1] Tel.: +49 611 9625716; fax: +49 611 9625257.

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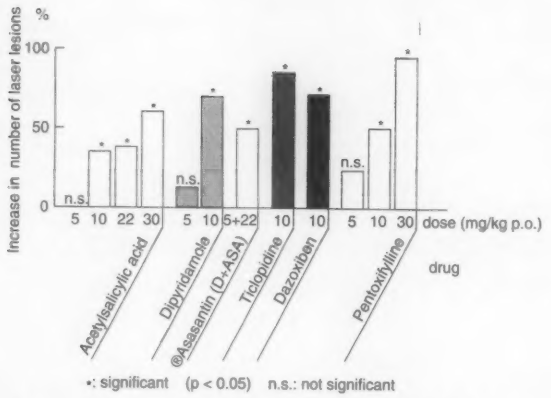

Fig. 1. Effects of several vascular agents on susceptibility of blood vessel endothelium to laser-induced thrombus formation.

The results demonstrate that thrombi form after localized endothelial cell damage, in the absence of damage to the basal lamina. It has also been shown that platelets and fibrinogen accumulate near the area of damage. PTX exhibits dose-dependent inhibition of mesenteric thrombus formation in rats and rabbits, with a significant antithrombotic effect observed in rats after a PTX dose of 10 mg/kg (Fig. 1).

In addition, studies on hamsters and rats have shown that PTX significantly inhibits oxLDL-induced leukocyte adhesion to the microvascular endothelium of arterioles [9], as well as LPS-induced leukocyte adhesion in venules (Fig. 2). PTX (3 mg/kg) also reduces endotoxin-induced rat endothelial permeability to macromolecules, as shown by the extravasation of fluorescently-labelled rat serum albumin (Fig. 3).

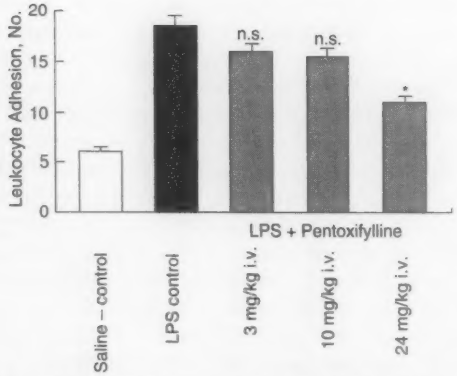

Fig. 2. Effects of pentoxifylline on endotoxin (lipopolysaccharide)-induced leukocyte adhesion in venules.

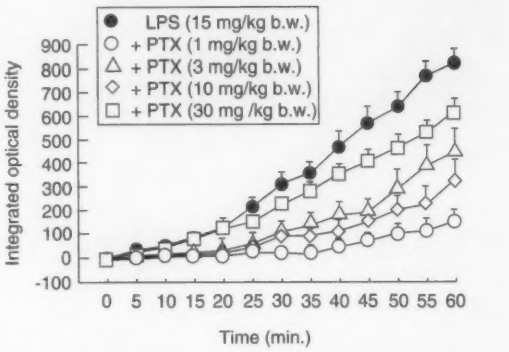

Fig. 3. Pentoxifylline (PTX) effect on endotoxin (lipopolysaccharide)-induced wall permeability in venules.

4. Summary

PTX counteracts the increased microvessel permeability which is a feature of the inflammatory aspect of atherogenesis. Furthermore, PTX inhibits oxLDL-induced leukocyte adhesion and, via its positive influence over fibrinolysis and anti-aggregatory factors (e.g. prostacyclin), inhibits thrombus formation. PTX thereby preserves the integrity of the walls of the microcirculatory blood vessels, while reducing the likelihood of thrombosis and its potentially life-threatening sequelae.

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Atherosclerosis 131 Suppl. (1997) S29–S31

ATHEROSCLEROSIS

Epidemiology of peripheral vascular disease

F.G.R. Fowkes [1]

Wolfson Unit for Prevention of Peripheral Vascular Diseases, Department of Public Health Sciences, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, UK

1. Introduction

Peripheral arterial disease (PAD) affects approximately 30% of older individuals in the general population (Fig. 1). The principal symptom, intermittent claudication, occurs only rarely in those under 50 years of age, but increases dramatically in older individuals. In the Edinburgh Artery Study, a random sample survey of the general population, the prevalence of claudication in men increased from 2.2% in the 50 to 59 year age group to 7.7% in the 70 to 74 year age group [1]. In most surveys, the prevalence is slightly higher in men than in women; however, this difference diminishes with age.

2. Assessment of asymptomatic disease

Recently, increasing effort has been made in the measurement of asymptomatic disease in the legs. Population-based studies have, for the most part, used the ankle brachial pressure index (ABPI), the ratio of ankle to arm systolic pressure, as an indirect indicator of asymptomatic disease. The results of these studies suggest that for every claudicant in the community, there are at least two individuals with asymptomatic disease of sufficient severity to produce a reduction in the ABPI [2]. The Edinburgh Artery Study, in which ABPI and a reactive hyperaemia test were used as measures of asymptomatic PAD, revealed that approximately 25% of the population aged between 55 and 74 years had findings suggestive of asymptomatic disease.

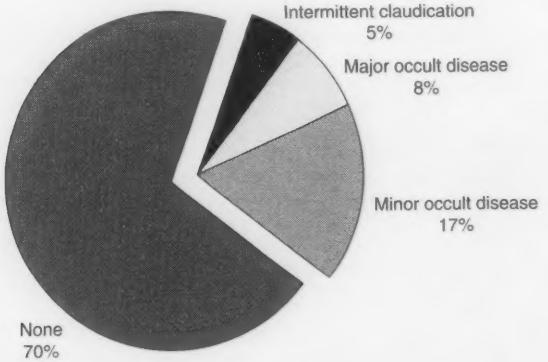

Fig. 1. Prevalence of peripheral arterial disease in the general population aged 55 to 74 years.

3. Co-incidence of peripheral arterial disease with coronary artery disease and thrombotic stroke

Since arterial disease in the legs, coronary artery disease and the majority of strokes represent manifestations of atherosclerosis, it is not surprising that the three conditions often occur together. The prevalence of coronary artery disease among claudicants in the general population is between two and four times higher than in non-claudicants. Around 50% of claudicants also suffer from angina and, conversely, patients with angina are six times more likely to have claudication (Speedwell Study) [3]. Furthermore, the Edinburgh Artery Study [1] and a longitudinal study (Malmo) [4] have shown that individuals with major asymptomatic PAD are twice as likely as normal subjects to suffer from concomitant coronary artery disease. These studies also showed that individuals with asymptomatic PAD had a two- to three-fold higher coronary event rate during 5 to 8 years of follow-up, compared with healthy controls [4,5].

[1] Tel.: +44 131 6503220; fax: +44 131 6506904.

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4. Risk factors for peripheral arterial disease

Cigarette smoking is an important risk factor for PAD. Among vascular patients attending hospital, more than 90% have a history of current or recent smoking [6]. It has been estimated that approximately half the occurrence of PAD in the community might be attributable to smoking [7], which would appear to have a greater effect than other risk factors. Smoking may, however, be more important in the development of PAD than of coronary artery disease—the risk of PAD being at least three-fold higher in smokers than in non-smokers [8].

The role of elevated blood pressure as a risk factor for PAD has been difficult to study, particularly as it is unclear whether some blood pressure elevation may be due to PAD itself, rather than vice versa. Hospitalized vascular cases tend to have higher mean blood pressures and prevalence of hypertension than healthy controls. In the general population, intermittent claudication has been shown to be related to elevations in both systolic and diastolic pressure [9], although a more consistent relationship exists with systolic pressure. For example, in the Edinburgh Artery Study [8] systolic blood pressure was significantly higher in individuals with both symptomatic and asymptomatic PAD than in normal subjects. Similarly, a US study has shown that hypertension imposed a three-fold increase in the risk of intermittent claudication at a 26-year follow-up (Framingham Study) [10].

The link between serum lipid levels and the risk of PAD has been the subject of several hospital studies [11] that have suggested that serum triglycerides may be more strongly related to PAD than to coronary artery disease, possibly because the most severe cases are identified in hospital. This raises the question of whether serum triglycerides exert a more profound effect in the population of patients with severe PAD and coronary artery disease. In the general population, claudicants have higher serum triglyceride and cholesterol levels and lower high-density lipoprotein cholesterol levels than non-claudicants [10]. However, some studies suggest that, as with coronary artery disease, serum triglyceride levels are not associated with risk for PAD after adjustment has been made for other lipids [8,12,13].

Diabetes mellitus is a recognized risk factor for critical limb ischemia and is related to the occurrence of milder forms of PAD. There is some indication, however, that contrary to observations made in hospital, diabetes mellitus is not much more strongly related to PAD than to coronary artery disease in the community [8]. Diabetes may be a risk factor for PAD because of other effects of the disease contributing to critical limb ischemia, e.g. peripheral neuropathy and microvascular disease. However, the relationship between diabetic status and PAD has been found to be independent of other risk factors, such as age, cigarette smoking, blood lipid levels, body mass index and alcohol intake [8]. This implies that diabetes is likely to be an independent risk factor for PAD.

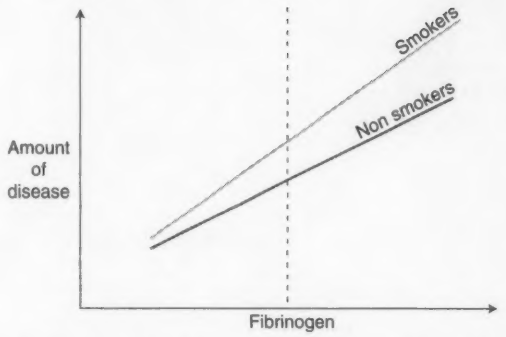

Fig. 2. The relationship between smoking, plasma fibrinogen level and peripheral arterial disease.

In addition to the classic coronary risk factors of smoking, hypercholesterolemia and hypertension, many other factors have been investigated as possible etiological agents for PAD. There is now strong evidence that plasma fibrinogen is related to both symptomatic and asymptomatic PAD in both smokers and non-smokers in the community [14] (Fig. 2). The Caerphilly and Speedwell Study [3] has shown that plasma fibrinogen is a risk factor for future intermittent claudication. Hypercoagulability, decreased fibrinolysis and increased blood viscosity may also be important [14–16] factors in the development of PAD and might be amenable to pentoxifylline intervention.

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Atherosclerosis 131 Suppl. (1997) S33–S34

ATHEROSCLEROSIS

Socioeconomic impact of peripheral vascular disease

Michael Drummond

Centre for Health Economics, University of York, Heslington, York YO1 5DD, UK

1. Introduction

In most developed countries, healthcare budgets account for a sizeable proportion of gross domestic product and have been subject to growing downward pressure. This has resulted in an increased level of interest in the socio-economic impact of disease and the economic evaluation of therapies, which involves a comparison of the costs and consequences of alternative treatments. Economic evaluation has now been applied widely to all forms of healthcare [1].

2. Burden of illness estimates in peripheral arterial disease

Economic burden of disease is typically considered to consist of three components: the direct costs, e.g. the resources used to treat the disease; the indirect costs, e.g. the loss of productivity of the patients or their carers; and thirdly, intangibles, i.e. the reductions in quality of life through pain or suffering associated with the disease.

To date, published burden of illness estimates have concentrated on critical limb ischemia (CLI) [2,3]. The main indices of burden included in the analysis by Hart and Guest [3] were direct costs: (i) the number and cost of general practitioner consultations and prescriptions; (ii) the number and cost of in-patient procedures; (iii) the amount and cost of long-term and community care services; (iv) the number and cost of hospital out-patient referrals; (v) the number and cost of diagnostic procedures; and (vi) the amount and cost of ambulance transport. They developed a treatment path model for CLI patients in the UK (Fig. 1), which formed the basis for estimating the total annual cost of CLI management (Fig. 2). This estimate allowed the conclusion that the future burden of CLI depends largely on factors affecting the requirement for reconstructive vascular surgery and amputation, which accounted for an estimated 56% of the total cost of CLI management. Therefore, effective non-surgical intervention in CLI, such as drug-based therapy, could produce marked savings for the healthcare services.

3. Economic evaluation of treatments in peripheral arterial disease (PAD)

As with studies of the burden of illness, the majority of economic evaluations of peripheral vascular disease consider the options for the treatment of more advanced disease, such as CLI. For example, Davies et al. [4] compared the cost-effectiveness of primary limb amputation and arterial reconstruction and found that

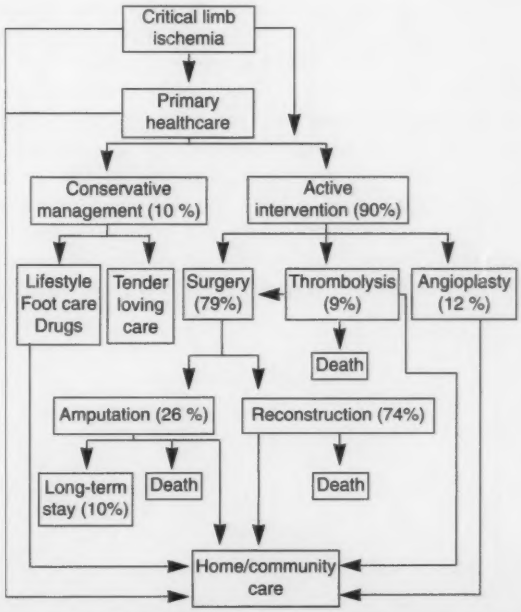

Fig. 1. Treatment path model for critical limb ischemia patients [3].

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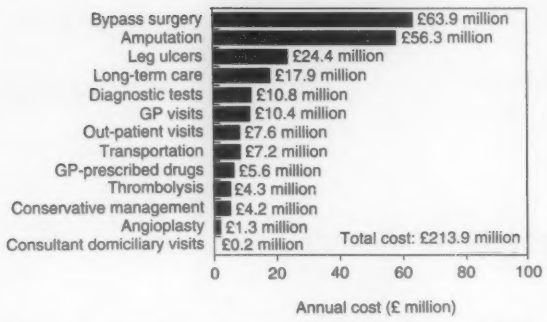

Fig. 2. Composition of the burden of illness for critical limb ischemia in the UK (1994 costs) [3]. Reproduced with permission of Brookwood Medical Publications, Ltd., UK.

the latter option resulted in lower overall costs. This was due to the relatively low use of rehabilitation and community care services by patients in the arterial reconstruction treatment group.

Hunick et al. [5] compared the relative benefits and cost-effectiveness of revascularization for femoropopliteal disease via percutaneous translumenal angioplasty or bypass surgery, using a decision analysis model based on data from the literature for mortality, morbidity, potency and cost. The conclusion was that, in patients with disabling claudication and femoropopliteal stenosis or occlusion and in patients with chronic critical ischemia with stenosis, angioplasty is the preferred initial interventional treatment. In contrast, bypass surgery was found to be the preferred initial treatment in patients with chronic critical ischemia and femoropopliteal occlusion.

In a comparison of thrombolytic (urokinase) therapy versus surgical intervention in acute peripheral arterial ischemia, limb salvage and survival rates were used as the primary endpoints [6]. Although cumulative limb salvage rates were the same for both treatment groups (82% at 12 months post-intervention), the cumulative survival rate was significantly greater in the group receiving thrombolysis (84% vs 58% at 12 months, $P = 0.01$). Furthermore, there was only a modest increase in hospital cost in the thrombolysis treatment group, compared to that of the surgical group (median $15 672 vs. $12 253, $P = 0.02$). It was concluded that intra-arterial thrombolytic therapy was associated with a reduced incidence of cardiopulmonary complications while in hospital and a corresponding increase in patient survival rate, without an appreciable increase in costs. Thrombolytic therapy may, therefore, represent a therapeutically and cost-effective alternative to surgery in the initial treatment of acute limb-threatening PAD.

While there are few published economic evaluations of drugs, such as pentoxifylline (PTX), which are indicated for use in the earlier stages of PAD, a study has been conducted to evaluate the Medicare care and cost outcomes of PAD treatment with PTX [7]. In this study, the use of a therapeutic dose of PTX for 120 days significantly reduced the requirement for PAD-related invasive therapy and diagnostic procedures, but did not lower the risk for PAD-related hospitalization and related care. Nevertheless, the high cost of managing the later stages of PAD suggests that effective early treatment could offer the potential for general resource savings, as well as improving the quality of life of the patient.

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Atherosclerosis 131 Suppl. (1997) S35–S36

ATHEROSCLEROSIS

Quality of life assessment in peripheral arterial disease

William R. Hiatt [1]

The Colorado Prevention Center, University of Colarado Health Sciences Center, 789 Sherman Street, Suite 200, Denver, CO 80203, USA

1. Introduction

Patients with symptomatic peripheral arterial disease (PAD) have a limited arterial supply which does not meet the metabolic demand of the muscles of the lower extremity, resulting in intermittent claudication during ambulatory activity. Claudication affects a large proportion of the middle-aged and elderly population, with an estimated prevalence of 3–7% in the general population. Many patients with claudication have severely limited walking ability, adversely affecting their social, leisure and occupational activities. In one study, up to 38% of patients under the age of 55 years seeking treatment for claudication were disabled and required public assistance [1]. These statistics have greatly increased the interest in the evaluation of the clinical efficacy of established therapies for PAD, such as exercise rehabilitation programs, surgery or angioplasty. In addition, there are several major ongoing clinical trials evaluating the efficacy of new pharmacological agents to treat claudication and improve functional capacity. Whether the patient is being considered for enrollment in a clinical trial, or is presenting to their physician for an approved therapy, it is critical to recognize that the primary treatment goals are the relief of claudication pain and an improvement in daily ambulatory activity. This would in turn be expected to produce an increase in the range of physical activities which the patient can perform. The severity of functional impairment at baseline and the response to therapy can be reliably evaluated using a number of instruments.

2. Approach to testing

Treatment decisions for patients with claudication should include the following two criteria: (1) patients selected for treatment should have a clinically significant degree of functional impairment as a result of claudication; and (2) clinicians should recommend only specific claudication therapies which have been scientifically demonstrated to improve functional status. Thus, the primary outcome measures of all claudication interventions should be patient-based and should be capable of demonstrating clinically relevant changes in functional status.

3. Laboratory assessment of claudication therapy

Historically, a number of laboratory-measured variables have been used to assess treatment results, including changes in peripheral hemodynamics, e.g. the ankle-brachial pressure index (ABPI), and treadmill exercise performance. The evaluation of changes in peripheral hemodynamics has been a primary means of assessing the technical success of bypass surgery or angioplasty. However, these tests do not correlate well with the degree of exercise impairment or with functional limitation in patients with claudication. In addition, a number of medical treatments can significantly improve treadmill exercise performance with no alteration in leg blood flow or ankle blood pressure. Also, some interventions may improve a hemodynamic endpoint independently of any meaningful clinical modification (e.g. angioplasty may augment ankle blood pressure, but may not improve functional status). Therefore, the monitoring of changes in peripheral hemodynamics alone is an inadequate measure of treatment success in patients with intermittent claudication.

A change in treadmill performance in response to therapy is the most commonly used endpoint in clinical claudication trials. The advantage of using the treadmill test to evaluate claudication treatment is that it provides reproducible objective data. It is, however, an expensive method and does not directly assess patient perception of functional impairment, nor whether therapy has resulted in benefit. In future clinical trials of

[1] Tel.: +1 303 8609900; fax: +1 303 8601288.

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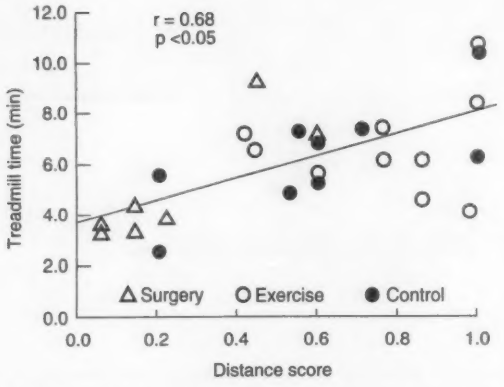

Fig. 1. Correlation between treadmill test score (time to claudication) and distance score derived from WIQ questionnaire [3].

claudication therapy, it has been suggested that laboratory assessments should serve only as secondary endpoints.

4. Questionnaire assessment of functional status

Questionnaires provide patient-based information on claudication severity and response to therapy. A comprehensive approach should be adopted to encompass a wide range of functional domains. For example, it would be expected that all claudication therapies would alleviate pain and improve walking capacity. Such an improvement could, but would not necessarily, lead to an improved quality of life.

The Walking Impairment Questionnaire (WIQ) has been developed and validated specifically in the claudication population and quantifies the ability to walk defined distances, at different speeds, and to climb flights of steps, as well as rating the severity of claudication pain [2]. It is therefore, a disease-specific instrument for use in the community setting. The WIQ has been validated by comparison with the graded treadmill test (Fig. 1) [3] which has been shown to give stable and reproducible results [4] and has been used extensively in clinical trials of claudication drugs (results not published).

The Physical Activity Recall (PAR) questionnaire provides a more global measure of physical activity, estimating total energy expenditure [5] and has been used primarily in large population studies of healthy individuals, as well as in diabetics [6]. The Medical Outcomes Study SF-36 questionnaire evaluates physical function and role limitations and has been extensively validated in patients with somatic and psychiatric conditions of varying severity [7]. In contrast to the WIQ and PAR, which measure only walking ability and physical activity, the SF-36 assesses multiple functional domains (Table 1).

Table 1
Parameters evaluated by the SF-36 questionnaire

Physical function
Social function
Role function
Overall health
Pain
Vitality
Health perceptions

In summary, the questionnaires described above represent essential elements in the accurate assessment of treatment outcomes in intermittent claudication, describing the overall impact of claudication and its treatment on health status.

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ATHEROSCLEROSIS

Clinical experience with pentoxifylline

Karl-Heinz Labs *, R. Labs, M. Rößner

Department of Clinical Angiology, Hoechst Marion Roussel Cardiovascular TA, Rheingaustraße 190–196, D-65174 Wiesbaden, Germany

1. Epidemiology and risk

Peripheral occlusive arterial disease (POAD) is a common disorder, with a prevalence in the elderly of approximately 10% [1]. POAD is one manifestation of generalized atherosclerosis with a co-morbidity between POAD and coronary artery disease (CAD) of 50–90% [1]. Asymptomatic but angiographically severe CAD is to be expected in approximately 30% of POAD patients [2]. Cardiac mortality in asymptomatic (Stage I) POAD is increased by a factor of 2 to 3 and will rise by an additional factor of 2 if the disease becomes symptomatic [3]. Cardiac mortality is also dependent on the degree of hemodynamic impairment which accompanies POAD. An assumed overall mortality of 5% in a general POAD population will fall to 3% in mildly diseased patients (ank'e/brachial pressure index [ABPI] > 0.5), but will rise to approximately 10 to 15% in individuals with an ABPI ≤ 0.5 [3,4]. Therefore, POAD has a high cardiac co-morbidity and mortality and can be used as a marker for asymptomatic CAD.

2. Claudication distance testing

In the clinical trial setting, both initial claudication distance (ICD) and absolute claudication distance (ACD) are routinely assessed by treadmill testing, using either a graded- or fixed-load treadmill protocol with a treadmill speed of 3.2 km/h (2 mph) and a gradient of 12%. These high speed and gradient values are used in order to reduce procedure-related variance and to guarantee a high test reproducibility (inversely correlated with applied work load per unit time). However, such treadmill settings are totally unphysiological, because no patient continuously walks at high speed (2 mph) up a steep (12%) slope. This means that if real-life conclusions are to be drawn from treadmill tested claudication distance changes, conversion factors must be applied. Since speed and gradient are independent variables, both must have a conversion factor (of 2–3) applied, yielding a total conversion factor of 4–6 [5–9]. Therefore, if a patient showed an improvement in ICD of 20 m on a treadmill at 3.2 km/h with a slope of 12%, the corresponding improvement in daily life walking ability would be 80–120 m. In the survey of pentoxifylline (PTX) effects on claudication distances described below, a conservative conversion factor of 2.5 has been chosen in order to ensure that the effects of PTX are not over-estimated.

3. Results with pentoxifylline

This survey focuses on pivotal trials of PTX in POAD Stage II (intermittent claudication) [10–12], with particular emphasis placed on the study by Lindgärde et al. [12]. The Porter et al. [10] and Lindgärde et al. [12] studies both fulfill all requirements of the 1996 European Union Guidelines [13], being double-blind, placebo-controlled, parallel group studies of 6 month duration. Both studies included a 4–6 week run-in period, during which stability of claudication distance had to be proven (i.e. ICD variance $\leq 25\%$). The 95% confidence intervals for the differences in results from the compared groups are shown in Fig. 1(a–c), from which two conclusions may be drawn. Firstly, all results (whether ICD, ACD or minimum improvement measured between weeks 16 and 24, which is the most conservative endpoint estimate possible) favored PTX and, secondly, the reproducibility of the individual study results was confirmed.

Using the conservative factor of 2.5 for the conversion of treadmill tested changes in claudication distance into values relevant to everyday life, the PTX group was found to exhibit increases in ICD and ACD of 88 and 112 m [10] and 154 and 165 m [12], respectively.

* Corresponding author. Tel.: +49 611 9625781; fax: +49 611 9629271.

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In addition to the intention-to-treat population (Fig. 1(a–c)), two sub-populations are of particular clinical interest: (i) patients with seriously limited walking ability, having the poorest prognosis and being candidates for reconstruction, and (ii) patients exhibiting the highest probability of a successful outcome with PTX treatment and thus presenting with a true indication for PTX.

In patients with a baseline ICD < 70 m, the improvement in claudication distance for PTX and for placebo were found to be 82 and 15 m [10] and 109 and 41 m [12], respectively (values converted to 'everyday' conditions). Using the more rigorous minimum improvement assessment, rather than using mean results, during study weeks 16–24, the following results were achieved from the Porter et al. study [10]: ICD (PTX-treated) 86 m, ACD (PTX-treated) 85 m, ICD (placebo) 23 m, ACD (placebo) 32 m. The results of the Lindgärde et al. study [12] were: ICD (PTX) 104 m, ACD (PTX) 104 m, ICD (placebo) 53 m, ACD (placebo) 35 m.

In the Scandinavian study [12], a responder or target population was defined on the basis of a duration of POAD symptoms of > 1 year and an ABPI ≤ 0.8. This population exhibited the best results with PTX treatment (Fig. 1(d)), as well as the greatest probability of a successful PTX treatment outcome. Converted to 'everyday' conditions, the PTX-induced increase in walking distances were 191 and 208 m for ICD and ACD, respectively, (placebo ICD: 95 m; placebo ACD: 118 m). The improvements in minimum distance walked for ICD and ACD were 130 and 139 m, respectively, (placebo ICD: 42 m; placebo ACD: 36 m). The magnitude of these improvements in walking distance may be regarded as being clinically relevant.

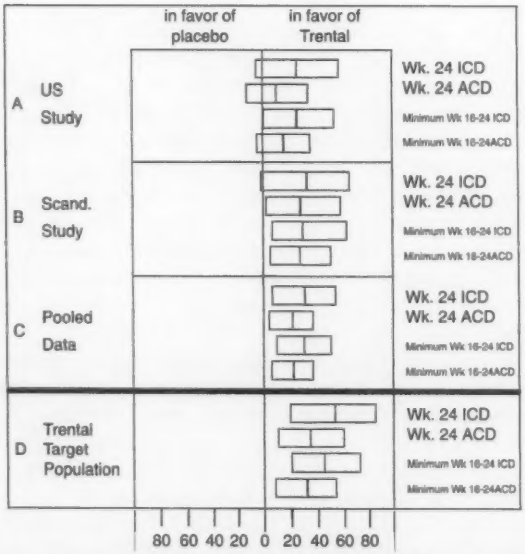
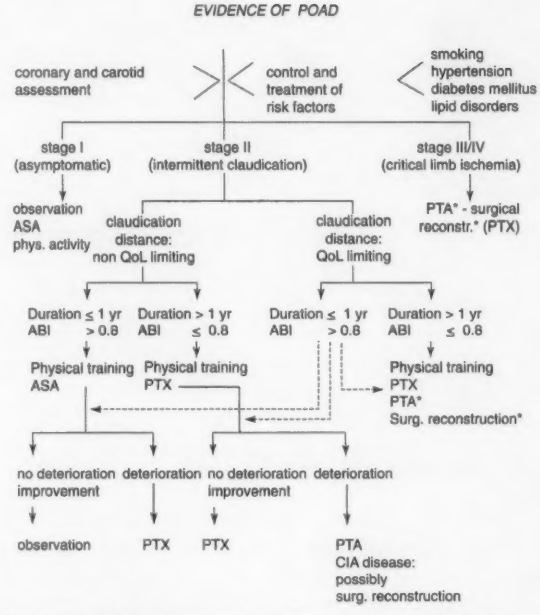


Fig. 1. Beneficial effect of Trental (pentoxifylline) on initial claudication distance (ICD) and absolute claudication distance (ACD) in US and Scandinavian studies (data from [10,12]).



ASA Acetylsalicylic acid
PTX Pentoxifylline
PTA Percutaneous Transluminal Angioplasty
CIA Common iliac artery
QoL Quality of life

* depending on:
- age
- degree of disability
- anatomical distribution of lesions
- concomitant diseases

---------- decision dependent on school of thought

Definition: non QoL limiting claudication distance: ICD and ACD limited due to POAD but without major influence on the patient's daily activities.

Fig. 2. A treatment algorithm for peripheral occlusive arterial disease.

The results of these two pivotal studies are confirmed by a number of smaller scale trials, 19 of which have been used in two summary analyses [14,15].

The results for PTX in Stage II POAD confirm: that statistically significant and clinically relevant improvements in walking distance are achieved; that this conclusion is also valid for patients with pronounced Stage IIb disease with claudication distances < 70 m; and that the combination of PTX plus exercise therapy is beneficial. Based on these findings and taking into account the current socio-economic environment, a treatment algorithm (Fig. 2) is suggested.

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ATHEROSCLEROSIS

Conclusions

Peripheral occlusive arterial disease (POAD) is a common disorder, which is particularly prevalent in the elderly, with an incidence of approximately 10%. It is but one manifestation of generalized atherosclerosis indeed, there is co-morbidity between POAD and coronary artery disease (CAD) (another product of the atherosclerotic process) of between 50–90%.

In POAD, the atherosclerotic plaque represents a protective, inflammatory-fibroproliferative response to a variety of insults, including smoking, hypercholesterolemia and hypertension. In addition to the role played by endothelial shear-stress, which can activate endothelial cells and thereby lead to increased cell replication, a number of blood cell responses are instrumental in the initiation and development of the atherosclerotic plaque, including the dynamic accumulation within the plaque of monocytes, activated macrophages and lipid-laden 'foam cells'. Activated macrophages generate numerous molecules which have the potential to inflict damage upon blood vessels. Central to this disease process is the production of reactive oxygen species, which can elicit the oxidation of low-density lipoproteins and increase vascular hyperpermeability. There is strong evidence to suggest that oxidized LDL (oxLDL) is a pivotal component of the atherosclerotic process, contributing in several ways to the progression of the disease. OxLDL is a chemoattractant for circulating monocytes and T lymphocytes, which make up a significant proportion of the cells in the plaque, and inhibits macrophage motility; it can therefore play an important role in the recruitment of cells into a plaque and thereby contribute to its enlargement. OxLDL also exhibits cytotoxicity towards vessel endothelial cells, further augmenting the atherogenic process by facilitating the migration of macrophages and additional LDL into the vessel wall. Therefore, the administration of compounds which can inhibit LDL oxidation, or which counteract adverse effects which may be elicited by oxLDL metabolites, may slow the progression of the atherosclerotic lesion. Growth factors generated by activated macrophages sequestered into the vessel wall also play a role in the disease, inducing smooth muscle cell proliferation and thereby enlarging lesions. Active macrophages may also release proteases, which themselves can damage structural components of the vessel and destabilize advanced plaques, leading to rupture, vessel thrombosis and life-threatening embolus formation.

In addition to the classic risk factors, such as elevated blood lipids and hypercholesterolemia, there is now strong evidence to suggest that plasma fibrinogen level is also related to the incidence of POAD, so that agents which lower plasma fibrinogen also have the potential to retard atherosclerosis.

As well as compounds which inhibit the effects of oxLDL and its products, agents which reduce blood viscosity via a down-regulation of leukocyte adhesion molecules or platelet aggregation factors and/or which reduce endothelial permeability, should also play a preventative role in atherosclerosis and help to retard the progression of conditions, such as POAD, which may be severely debilitating to the sufferer. The identification of existing agents which possess these specific properties, or the development of novel agents fulfilling these criteria, should expand our existing armoury against athersclerosis in all its manifestations.

Geert W. Schmid-Schönbein and Seymour Glagov

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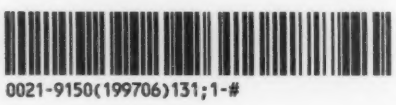

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